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APPLICATION FOR
PATENT OF INVENTION

(21)

N^o 81 23069

(54) **Metrological Analysis Device and Automatic Alarm in the event of Pollution, based on
the Use of a Biosensor and its Telemetry System.**

(51) **International classification (International Class^o). G 01 N 33/18, 23/00.**

(22) **Date of application 10th December 1981.**

(33) (32) (31) **Priority claimed:**

(41) **Date on which the application was made available
to the public Official Journal for Industrial Property -
"Lists" N^o 24 of 17th June 1983.**

(71) **Applicant: HUVE Jean-Louis. - FR.**

(72) **Invented by: Jean-Louis Huvé.**

(73) **Patentee: Idem (71)**

(74) **Representative:**

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Purchase of specifications at the National Printing Office, rue de la Convention - 75732 PARIS CEDEX 13**Best Available Copy**

The invention is based on the use of common rainbow trout as a high-resolution biosensor adapted for monitoring industrial waters.

In its general principle, the invention is not novel since a patented Troutometer currently exists on the market which has been used by INRA and a Reservoir Agency.

However, this method - based on the behavioural reactions of the fish - remains very crude. In the event of pollution, it does not provide any qualitative, let alone quantitative, response.

By contrast, the invention - the result of 10 years of research into the fundamental neurophysiology of trout - allows, by the methodology and technology it employs, a level of resolution and speed of response which are highly advantageous when detecting a certain number of synthesis molecules (comparable to micro-pollutants) in a fresh water or marine environment.

Regarding the methodological plan: the invention differs from the Troutometer since the pollution information is captured at the level of the olfactory nerve structures of the fish. This then allows a high resolution sensitivity over a large spectrum of chemical families on the one hand, and on the other allows the hardly quantifiable behavioural fluctuations - on which the current Troutometer is based - to be avoided.

Regarding the technological plan: the animal is the biosensor of a measurement system. It therefore ensures the following transduction function: chemical information on micro-pollution ----> particular electrical activity at the level of the nerve structures. It is sufficient to capture this electrical activity and utilise it.

Implementation

The bioelectric information present on the olfactory bulb is extracted by two platinum electrodes having a diameter of 3/100. It is amplified, then coded, and finally emitted at a centre frequency of 100 KHz by the emitter (EM, Figure 1/1). This CMOS-enabled emitter allows the changes in the electrical activity of the olfactory bulb to be code-converted exactly. The telemetry unit is fed by a lithium battery which (given the low overall consumption of the emitting portion) guarantees 1800 hours of autonomous running.

The trout (T, Figure 1/1) is placed in an aquarium tank (B, Figure 1/1), measuring 60x60x40 cm and fed with water, for monitoring.

The telemetry signal emitted by the emitter is captured by two stainless steel electrodes formed as plates (E_1 , E_2 , Figure 1/1) placed on the bottom and near the surface of the aquarium, respectively. This signal is then amplified by an amplifier (A, Figure 1/1) having 80 db gain in the 110-80 Hz band. The decoder (D, Figure 1/1) allows the high frequency signal to be demodulated, so as to obtain the biological signal initially present at the level of the olfactory bulb. This signal (electrobulbogram or EBG) is then processed by a fast Fourier transformation (FFT) by an inexpensive calculator (C, Figure 1/1). An FFT is therefore obtained which represents the distribution of energy as a function of frequency. In this case, said FFT is in the 0 to 25 Hz band (FFT, Figure 1/1). In the absence of any contamination, the spontaneous energy spectrum (sp, Figure 1/1) is distributed between 0 and 25 Hz, with a predominance between 2 and 12 Hz. The presence of a micro-pollutant changes this distribution and a different spectrum is obtained (mp, Figure 1/1). These changes in the spectral energy are processed by a correlator which allows the type of micro-pollutant and its concentration in the environment (concentration, chemical family, Figure 1/1) to be identified, as a function of the changes in the spectrum.

System characteristics

Resolution by mass: currently 10 ng/litre, i.e. 10 times greater than analysis by gas phase chromatography.

Distinguishing between products: relatively good and basically dependent on the type of correlation program.

Response times

Two aspects need to be differentiated:

- the physiological response time which is of the order of 500 ms from when the micro-pollutant touches the olfactory slits;
- calculated time: currently, the programs are not yet optimised. Three minutes are required for the correlation to be obtained.

Costs of the telemetry system:

comprising the animal, the emitter, the amplifier and the decoder: less than 5000 francs as of 1st December 1981.

Reliability of the telemetry system:

- no incidents regarding the electronics;

- regarding the surgical aspect of implantation: excellent physiological defence by the animal. No post-operative infection has yet been recorded, since surgery was performed in a sterile environment.

Using modest means, the proposed invention enables the monitoring of water intended for consumption or of seawater to be very correctly ensured. In the latter case, it is entirely possible to use trout, though this necessitates a period of three weeks to a month for the animal to adapt to the particular saline conditions of the environment. It is preferable to use a marine species (currently in the course of experimentation).

This invention, based on the principle of the biosensor, allows the physicochemical changes in the water to be monitored long-term.

The overall response time of the analysis system is interesting (clearly shorter than with the classic physicochemical analysis methods). The reliability of the system is acceptable.

It is conceivable that a systematic industrial application may be considered, for solving water pollution problems

- upstream of the water intake intended for consumption;
- downstream of purification plants;
- and generally wherever the chemistry of water is to be monitored.

CLAIMS

1. A device which allows micro-pollutants to be detected in freshwater or in seawater, based on the use of a biotelemetry system, characterised by the utilisation of the sensory capacity of a biosensor-type animal (T) living in a tank (B).

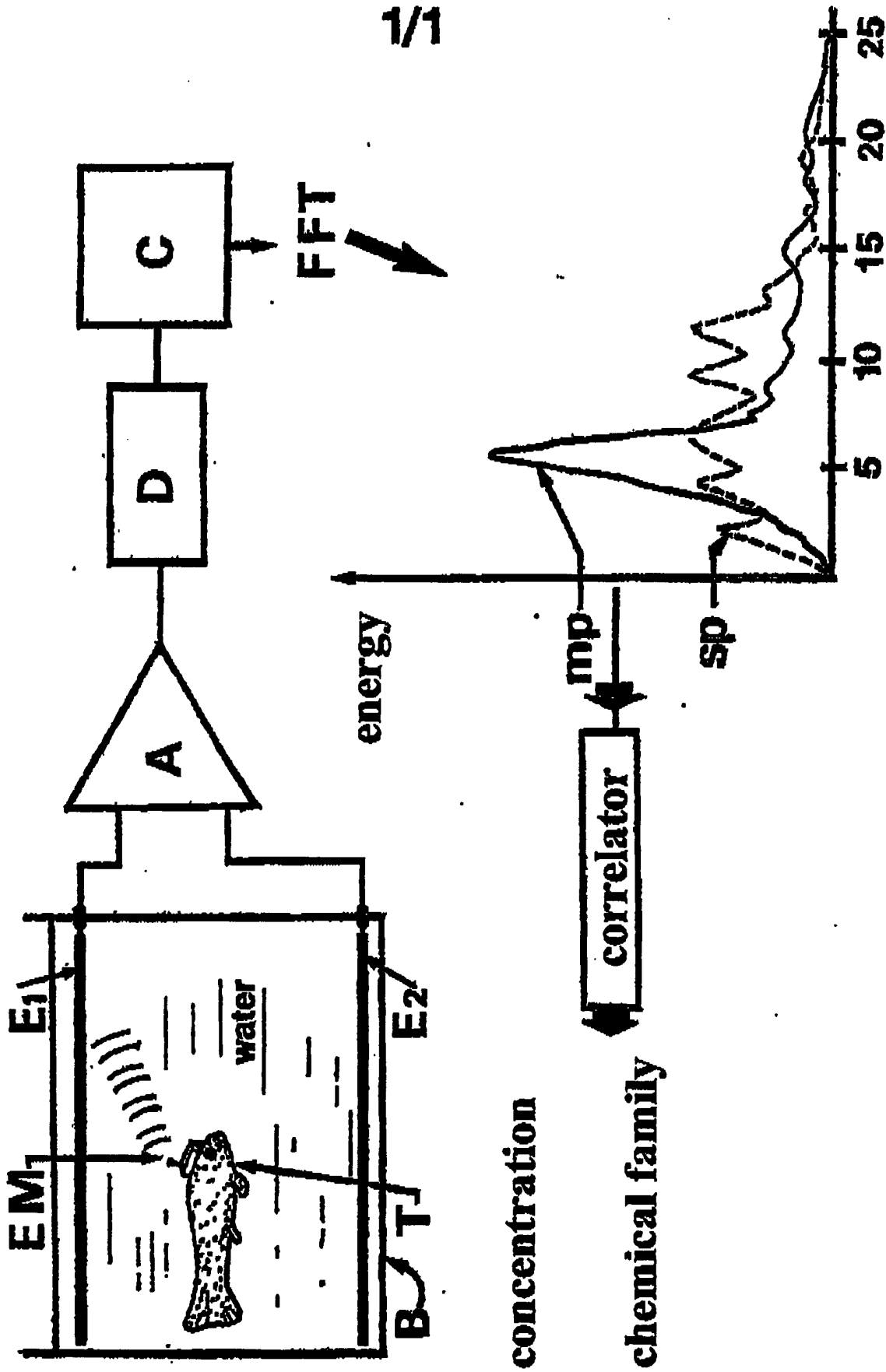
2. The device according to claim 1, characterised by the presence of an emitter, permanently implanted surgically in the cranium of the animal (EM).

3. The device according to claim 2, characterised by using electrodes (E_1 and E_2) to capture the emission of a bioelectric signal emitted by the nerve structures of the animal.

4. The device according to claim 1, characterised by the presence of an amplification (A) and decoding (D) unit.

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**DEMANDE
DE BREVET D'INVENTION**

(21)

N° 81 23069

(54) Dispositif métrologique d'analyse et d'alarme automatique en cas de pollution basé sur l'utilisation d'un biocapteur et de sa chaîne de télémesure.

(51) Classification internationale (Int. CL⁸). - G 01 N 33/18, 23/00.

(22) Date de dépôt..... 10 décembre 1981.

(33) (32) (31) Priorité revendiquée :

(41) Date de la mise à la disposition du
public de la demande..... B.O.P.I. — « Listes » n° 24 du 17-6-1983.

(71) Déposant : HUVE Jean-Louis. — FR.

(72) Invention de : Jean-Louis Huvé.

(73) Titulaire : *Idem* (71)

(74) Mandataire :

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L'invention consiste en l'utilisation de la truite commune arc-en-ciel comme biocapteur à haute résolution adapté à la surveillance des eaux de consommation.

Dans son principe général, l'invention n'a rien de nouveau puisqu'il existe actuellement sur le marché un Truitemètre breveté qui a été mis au point par l'INRA et une Agence de Bassin.

Cependant ce procédé, basé sur les réactions comportementales du poisson, reste très grossier. Il ne donne, lors d'un incident de pollution, aucune réponse qualitative et encore moins quantitative.

10 L'invention par contre, résultat de 10 années de recherche en neuro-physiologie fondamentale sur la truite, permet de par la méthodologie et la technologie mises en oeuvre un niveau de résolution et une rapidité de réponse tout à fait intéressants lorsqu'il s'agit de détecter un certain nombre de molécules de synthèse (assimilables aux micropolluants) en mi-
15 lieu d'eau douce ou en milieu marin.

Sur le plan méthodologique : l'invention se distingue du Truitemètre puisque l'information de pollution est recueillie au niveau des structures nerveuses olfactives du poisson. Ceci permet donc d'une part une haute finesse de résolution sur un large spectre de familles chimiques, et
20 d'autre part de se dégager des fluctuations comportementales peu quantifiables sur lesquelles est basé le Truitemètre actuel.

Sur le plan technologique : l'animal est le biocapteur d'une chaîne de mesure. Il assure donc la fonction de transduction suivante :
information chimique de micropollution —> activité électrique particulière
25 ra au niveau des structures nerveuses. Il suffit de recueillir cette activité électrique et de l'exploiter.

Mise en oeuvre

Les informations bioélectriques disponibles sur le bulbe olfactif sont prélevées par 2 électrodes de platine de 3/100 de diamètre. Elles sont amplifiées puis codées, enfin émises sur une fréquence centrale de 100 KHz par
30 l'émetteur (EM figure 1/1). Cet émetteur réalisé en technologie CMOS permet un transcodage précis des modifications d'activité électrique du bulbe olfactif. L'alimentation de l'unité téléométrique s'effectue par pile lithium ce qui garantit (compte tenu de la faible consommation globale de la
35 partie émission) une autonomie de 1800 heures.

La truite (T figure 1/1) est placée dans un bac (B figure 1/1) de type aquarium de 60x60x40 cm alimenté par l'eau à surveiller.

Le signal téléométrique issu de l'émetteur est recueilli par 2 électrodes

d'inox en forme de plaque (E_1, E_2 fig. 1/1) placées respectivement au fond de l'aquarium et près de la surface. Ce signal est ensuite amplifié par un amplificateur (A fig. 1/1) ayant 80 db de gain dans la bande 110-80 Hz. Le décodeur (D fig. 1/1) permet de démoduler le signal haute fréquence et d'ob-

5 tenir ainsi le signal biologique initialement présent au niveau des bulbes olfactifs. Ce signal (Electrobulbogramme ou E.B.G.) est ensuite traité par transformée de Fourier rapide (F.F.T.) (terminologie anglo-saxonne) par un ordinateur à faible coût (C fig. 1/1). On obtient donc une F.F.T. qui représente la répartition de l'énergie en fonction de la fréquence.

10 Ici cette FFT est réalisée dans la bande 0 + 25 Hz (FFT fig. 1/1).

En l'absence de toute contamination le spectre énergétique spontané (Sp fig. 1/1) se répartit entre 0 et 25 Hz avec une prédominance entre 2 et 12 Hz. L'apparition d'un micropolluant modifie cette répartition et l'on obtient un spectre différent (mp fig. 1/1).

15 Ces modifications de l'énergie spectrale sont traitées par un corrélateur, ce qui permet de distinguer, en fonction des modifications du spectre, le type de micropolluant et sa concentration dans le milieu (concentration, famille chimique fig. 1/1).

Qualités du système

20 Résolution en masse : actuellement 10 ng/litre, c'est-à-dire 10 fois au-dessus de l'analyse par chromatographie en phase gazeuse.

Discrimination entre produits : relativement bonne et essentiellement dépendante du type de programme de corrélation.

Temps de réponse

25 Il faut distinguer deux aspects :

- le temps de réponse physiologique qui est de l'ordre de 500 ms dès que le micropolluant touche les fossettes olfactives.
- le temps de calcul : actuellement les programmes ne sont pas encore optimisés. Il faut 3 minutes pour que la corrélation soit obtenue.

30 Coût de la chaîne téléométrique :

comprenant l'animal, l'émetteur, l'amplificateur, le décodeur : inférieur à 5000 Fr à la date du 1/12/81.

Fiabilité du système téléométrique

- aucun incident en ce qui concerne l'électronique,
- 35 - en ce qui concerne l'aspect chirurgical de l'implantation: excellente défense physiologique de l'animal. Aucune infection post-opératoire n'a encore été constatée alors que les interventions ont lieu en milieu septique.

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L'invention qui est proposée permet, avec des moyens modestes, d'assurer très correctement la surveillance de l'eau destinée à la consommation ou de l'eau du milieu marin. Dans ce dernier cas l'utilisation de la truite est tout à fait possible mais nécessite une période d'adaptation de 3 semaines à un mois de l'animal aux conditions particulières de salinité du milieu. Il est préférable d'utiliser une espèce marine (actuellement en cours d'expérimentation).

Cette invention qui est basée sur le principe du biocapteur permet donc une surveillance des modifications physico-chimiques de l'eau au sens large du terme.

Le temps de réponse global de la chaîne d'analyse est intéressant (nettement plus court que par les procédés physico-chimiques classiques d'analyse). La fiabilité du système est acceptable.

On peut considérer qu'une application industrielle systématique peut être envisagée pour répondre aux problèmes de pollution de l'eau

- en amont des prises d'eau destinées à la consommation,
- en aval des stations d'épuration,
- et d'une façon générale lorsqu'il s'agit de surveiller la chimie d'une eau.

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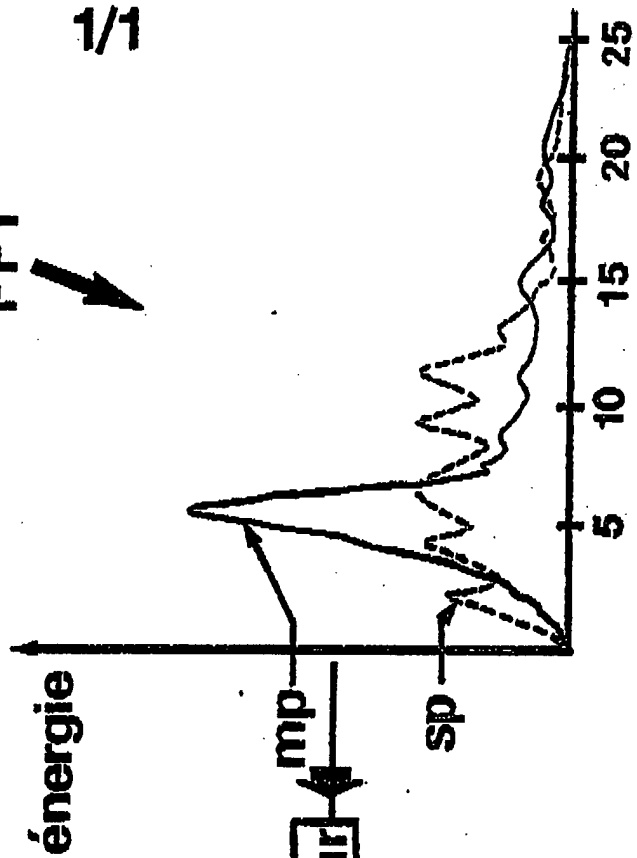
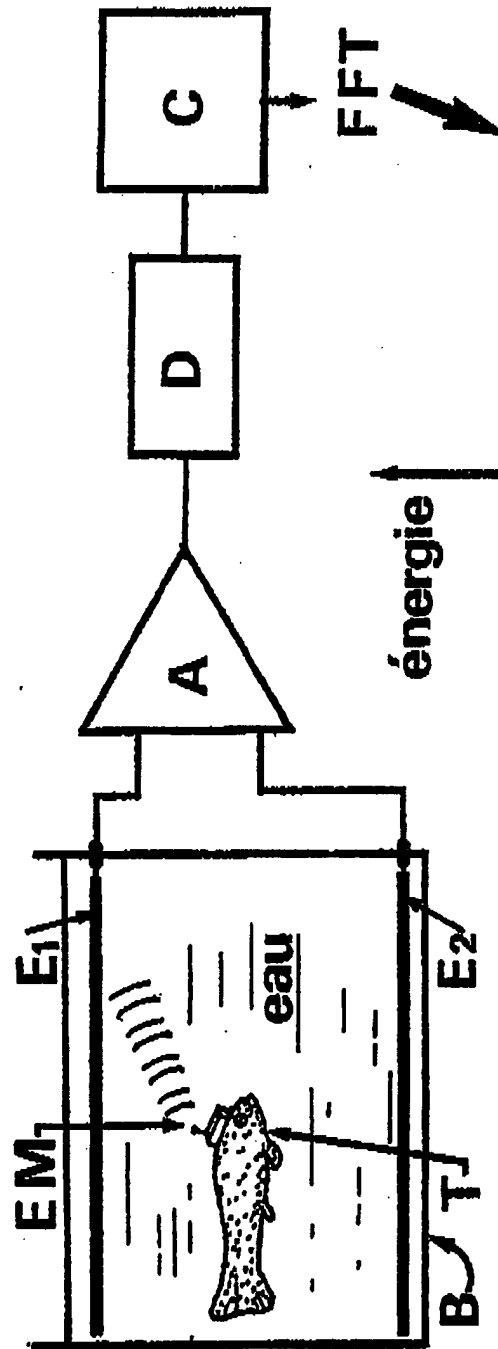
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REVENDICATIONS

1. Dispositif permettant la détection de micropolluants dans l'eau douce ou dans l'eau de mer, basé sur l'utilisation d'une chaîne de biotélémessure, caractérisée par l'exploitation de la sensorialité d'un animal de type biocapteur (T) vivant dans un bac (B).
- 5 2. Dispositif selon la revendication 1, caractérisé par la présence d'un émetteur implanté chirurgicalement à demeure sur le crâne de l'animal (EM).
3. Dispositif selon la revendication 2, caractérisé par le recueil de l'émission d'un signal bioélectrique issu des structures nerveuses de
10 l'animal par les électrodes (E_1 et E_2).
4. Dispositif selon la revendication 1, caractérisé par la présence d'une unité d'amplification (A) et de décodage (D).

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corrélateur

concent.

famille ch.



P.B. 5819 • Patanilaan 2
3200 HV Rijswijk (ZH)
T +31 70 340 3040
TX 31651 epo nl
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Marx, Lothar, Dr.
Patentanwälte Schwabe, Sandmair, Marx
Stuntzstrasse 16
81677 München
ALLEMAGNE

Datum/Date

04. 02. 2005

Zeichen/Ref./RM.

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Anmeldung Nr./Application No./Demande n°/Patent Nr./Patent No./Brevet n°

01941230.3-2313/JP0105426

Anmelder/Applicant/Demandeur/Patentinhaber/Proprietor/Titulaire

MATSUSHITA ELECTRIC INDUSTRIAL CO., LTD.

COMMUNICATION

The European Patent Office herewith transmits the supplementary partial European search report under Rule 46(1) EPC relating to the above-mentioned European patent application.

Copies of the documents cited in the search report are enclosed.

The applicant's attention is drawn to the following:

The search Division informs the applicant that if the European search report is also to cover inventions other than the inventions first mentioned in the claims, a further search fee must be paid for each of these inventions, within ONE MONTH after notification of this communication.

If the application has been filed up to 30 June 1999, the search fee in force before 01 July 1999 (EUR 869.-) or the equivalent applicable on the date of payment is payable.

This applies also to the search fees requested under Rule 46(1) EPC.

See also OJ EPO 06/1999, 405.

Moreover, the Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims; reference is made to sheet C, which is attached to the search report.

☒ Additional set(s) of copies of the documents cited in the European search report is (are) attached as well.



Note to users of the automatic debiting procedure:

Unless the EPO receives prior instructions to the contrary, the search fee(s) will be debited on the last day of the period for payment. For further details see the Arrangements for the automatic debiting procedure, Supplement to OJ EPO 02/2002.

REGISTERED LETTER



European Patent
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**SUPPLEMENTARY
PARTIAL EUROPEAN SEARCH REPORT**
under Rule 46, paragraph 1 of the European Patent
Convention

Application Number

EP 01 94 1230

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	FR 2 518 265 A (HUVE JEAN LOUIS) 17 June 1983 (1983-06-17) * page 1, line 28 - line 29; figure 1 *	1, 2, 5, 8	A61B5/0484 A61B5/0478 A61N1/05 A01K67/00 G01N33/50 G01N33/15
			TECHNICAL FIELDS SEARCHED (Int.Cl.7)
			A61B G01N A01K
LACK OF UNITY OF INVENTION			
<p>The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:</p> <p>see sheet B</p> <p>The present partial European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims.</p>			
Place of search		Date of completion of the search	Examiner
Munich		18 January 2005	Pille, S
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons</p> <p>& : member of the same patent family, corresponding document</p>			



European Patent
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**LACK OF UNITY OF INVENTION
SHEET B**

Application Number
EP 01 94 1230

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

1. claims: 1-8

screening olfactory mucosa stimulating compounds

2. claim: 10

treatment apparatus

3. claims: 11-28

measuring electrode portion

The search division considers that there are 3 groups of inventions claimed in the present application:

SEARCHED	1) Claims 1-8
NOT SEARCHED	2) Claims 10
NOT SEARCHED	3) Claims 11-28.

The application lacks unity within the meaning of Article 82 EPC for the following reasons:

The common concept linking together the independent claims 1, 10 and 11 is a measuring electrode portion implanted in an olfactory bulb of an organism.

This common concept is not novel, see document EP970702 (par. 47), cited in the ISR.

It appears that with regard to existing prior art, the special technical features of the first group relate to the administration and analysing means.

The special technical features in the second group are to be found in the means for supplying a stimulation pattern.

The special technical features in the third group are the plurality of micro electrodes and their arrangement.

The three claimed groups of inventions appear to be applicable independently of each other and solve different problems (i.e. screening of olfactory mucosa stimulating compounds vs. treatment by means of supplying stimulation patterns vs. improved measuring electrode).

The subject-matter of the three groups, defined by the problems posed and the solutions proposed, are thus so different from each other that no technical relationship or interaction can be appreciated so as to be linked as to form a single general inventive concept.



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**INCOMPLETE SEARCH
SHEET C**

Application Number
EP 01 94 1230

Claim(s) searched completely:
1-8

Claim(s) not searched:
9, 29-31

Reason for the limitation of the search (non-patentable invention(s)):

Article 52 (4) EPC - Method for treatment of the human or animal body by therapy

Further limitation of the search

Claim(s) not searched:
9, 29-31

Reason for the limitation of the search:

Claim 9: no specific constitution of an olfactory mucosa stimulating compound is described (see also International Search Report)

**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

EP 01 94 1230

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
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18-01-2005

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
FR 2518265 A	17-06-1983	FR 2518265 A1	17-06-1983

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